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Ecology of Archaebacteria from Extreme Environments and the Initial Microfouling Community by Biosignatures.

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Dr. David C. White

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19. ABSTRACT (Continue on reverse if necessary and identify by block number)

The research program represented a consolidation of three research programs 1) the examination of the lipid metabolism of the Archaebacteria from the hydrothermal vents, 2) the analysis of the initial microfouling community using ultrasensitive signature lipid biomarker techniques, and 3) the development of an assessment program for the detection of microbial biofouling on painted surfaces for the Antifouling Coatings Development Program. Significant progress was amde in detecting and monitoring the hydrothermal vent microbiota from in situ samples and in bioreactors. New methodologies for significantly reducing chemical noise were developed for assessing the initial microfouling film and a test system for detecting biofilm formation and sublethal toxicity on the test surfaces with the initial microfouling organism Pseudomonas atlantica.

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Contract #: N0014-89-J-3095

Principal Investigators: David C. White, James B. Guckert

Institute: Institute for Applied Microbiology, University of Tennessee

Contract Title: Ecology of Archaebacteria, initial microfouling community, and antifouling coatings development program.

Period of Performance: 05-01-89 through 06-01-91

Objectives:

1. To develop and analyze the ether lipid components of the Archaebacteria from the hydrothermal vents.

2. To develop sensitive methods for analysis of signature biomarkers for the initial microfouling community.

3. To develop an assay system for the formation and toxicology of the microbial fouling community on the surfaces of developed in the antifouling coatings program.

Accomplishments:

1. We were able to develop a chromatographic method for the separation and quantitative analysis of the ether lipids from Archaebacteria. These lipids which consist of diphytanyl- and bidiphytanyl-glycerol ether derivatives are too high molecular weight to be made volatile at the temperatures that are possible for gas chromatography. D. B. Hedrick developed a method based on supercritical fluid chromatography (SFC) with supercritical carbon dioxide with a pressure gradient where he was able t separate the large molecular weight alcohols formed after the acid methnolysis of the lipids. These were then separated by capillary chromatography and detected by flame ionization detection. The analysis allowed us to show that there are diphytanyl glycerol ethers (DPE) and bi-diphytanyl glycerol ethers (BGE) in the neutral lipid, glycolipid, and polar lipid fraction. We were able to account for low levels in some Archaebacteria by showing that in these organisms the lipids are not extractible until **after** acid methanolysis (1). Using this technique it was possible to develop a rapid method for distinguishing the ether lipids utilizing FT/IR (2).

2. The principal problem with the ultrasensitive analysis of polar lipid biomarkers for very sparse microbial biofilms such as the initial microfouling community is the contamination of the solvent used for extraction with fatty acids. This leads to chemical noise. To decrease this problem we developed a microtechnique where we utilize a very small volume 5 ml total of extract and microcolumns for extraction. This was then further

improved by a "reverse serial extraction technique" in which the same small volume of extract is repeatedly exposed to biofilm samples. The development of a o-ring device based on a joint that is clamped to the surface for recovery of the contents inside the extractor allowed the recovery of multiple samples of biofilm from the same coupon. With this technique and the utilization of single ion extraction software in the gas chromatograph/mass spectrometer (GC/MS) it was possible to detect sub picomolar quantities of microbes in biofilms. This technique has been published in relationship to the recovery of microbes from the deep subsurface (3). With this technique and new developments it proved possible to show absolute correspondence between the phylogenetic relationships based on 16S RNA sequences and the cluster analyses of the phospholipid ester-linked fatty acids (4).

3. To evaluate the microfouling potential of newly developed antifouling coatings we developed a marine bacterial challenge assay. This assay was designed to test both the microfouling potential as well as the toxicological effects with respect to the bacterial physiological stress associated with exposure to any leached materials. This challenge assay can be expanded to include a marine diatom to provide an algal/bacterial challenge assay. The system developed involved an oligotrophic, continuous-flow (approximately 5 ml/min) flow of ASTM seawater with very dilute nutrients containing an inoculum of Pseudomonas atlantica, a gram-negative bacteria like those in the initial microfouling community. With this system the U.S. Navy standard F121 copper based paint was used and compared to stainless steel surfaces. Although the F121 paint gave off between 4 -8 mg Cu/L the bulk phase microbial densities remained at 3×10^6 and the biofilms developed to densities of 10^9 cells/sq. cm. The test system also allows the detection of sublethal toxicity as the o-ring extraction system allows recovery of the organisms which are then extracted and tested for the proportions of cyclopropane and trans monoenoic phospholipid ester-linked fatty acids. Both these accumulate in P. atlantica that are exposed to toxic stimuli. This test system in the presence of toxins such as mercury show these changes.

Significance:

In this research program the methods and techniques necessary to study the ecology of the hydrothermal vent extreme thermophilic Archaebacteria were developed. The test system using the P. atlantica challenge assay system was developed and shown to be effective in testing the anti-fouling coatings developed in the ONR program.

Future Programs:

Research based on the data and techniques developed in this test system have led to the development of an on-line test system utilizing the bioluminescent bacteria.

Publications:

1. Hedrick, D. B., J. B. Guckert, and D. C. White. 1991a. Archaebacterial ether lipid diversity analyzed by supercritical fluid chromatography: Integration with bacterial lipid protocol. J. Lipid Research 32: 659-666.

2. Hedrick, D. B., D. E. Nivens, C. Stafford, and D. C. White. 1991b. Rapid differentiation of Archaebacteria from eubacteria by diffuse reflectance Fourier-transform infrared spectoscopic analysis of lipid preparations. J. Microbiol. Methods 13: 67-73.

3. White, D. C., D. B. Ringelberg, J. B. Guckert, and T. J. Phelps. 1991. Biochemical markers for in situ microbial community structure. In Proceedings of the First International Symposium on Microbiology of the Deep Subsurface, (C. B. Fliermans and T. C. Hazen, eds.). January 15-19, 1990, Orlando, FL. WSRC Information Services, Aiken, SC. pp. 4-45 to 4-56.

4. Guckert, J. B., D. B. Ringelberg, D. C. White, R. S. Henson, B. J. Bratina. 1991. Membrane fatty acids as phenotypic approach to taxonomy of methylotrophs within the proteobacteria. J. Gen. Microbiol. 137: in press.